

NTP Research Concept: Valerian (*Valeriana officinalis*)

Project Leader:

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Nomination Background and Rationale:

Valerian (*Valeriana officinalis* [*V. officinalis*]) was nominated by the National Institute of Environmental Health Sciences for comprehensive toxicological characterization based on its widespread use in dietary supplements, the lack of adequate toxicological data, and concerns regarding potential adverse developmental and reproductive effects, particularly for women of child-bearing age. Valerian most commonly refers to extracts of the underground rhizomes and roots, including tinctures, essential oils, terpenes, terpene-free fractions, and residues from the species *V. officinalis* [CAS No. 8057-49-6] and its subspecies, which sometimes exclude *officinalis* from the name. Valerian oil [CAS No. 8008-88-6], as used in the U.S. and European official pharmacopoeias, also refers to *V. officinalis*. Valerian is an herbaceous perennial plant that is native to Europe and parts of Asia. Valerian products are sold as valerian alone or in combination with other herbs and supplements. Valerian is classified as a "dietary supplement" under the Dietary Supplement Health and Education Act of 1994.

Constituents. Valerian is readily available as a powder, tea, tincture, essential oil and root extracts. More than 150 compounds have been reported in the essential oil. It has several components that have some pharmacological actions. Constituents of valerian, include monoterpenes, sesquiterpenes, alkaloids, caffeic acid derivatives, valepotriates, flavonoids, lignans, and amino acids. A number of sesquiterpenoids, including bornyl acetate, isovalerate and valerenic acid, make up approximately 10-40% of the essential oil. Valepotriates are epoxy iridoid esters that are found up to 2% in the dried root. Valepotriates are not readily absorbed because they are rapidly degraded in acid to baldrinal and homobaldrinal.

There are several preparations of valerian. Initially, extracts are prepared by soaking the dried valerian root and/or rhizome in a solution followed by centrifugation and drying of the extract to concentrate the mixture. The extraction solutions range from water alone to various ethanol:water mixtures. The use of the different solutions results in very different valerian products. For example, valerenic acids are extracted in at least 30% ethanol, while valepotriates require at least 70% alcohol.

Use and dosage. Valerian is used to treat a variety of neurological and/or psychological ailments including insomnia, mood disorders, anxiety, and psychological stress conditions. It is also used in the treatment of menstrual cramps and menopausal symptoms. Recommended doses of the powders are as high as 2-3g up to 3 times a day. Extracts are often standardized to 0.3-0.8% of either valerenic or valeric acid (Natural Standard Monograph, 2008). Recommended doses of the tincture are typically 2-5 ml (1:5 w:v in 70% ethanol) several times/day.

Valerian is ranked as the 11th top selling botanical dietary substance in the United States (HerbalGram, 2009). Kennedy (2005) analyzed the National Health Interview survey conducted by the National Center for Health Statistics and found that 5.6% of the respondents reported taking valerian in the last 12 months. Stasio et al (2008) reported that in a survey of 201 college students from a private southeastern college, 9.5% of the students reported taking valerian for anxiety. These data suggest that valerian use is widespread and there is potentially significant exposure to women of reproductive age.

Human Studies:

Most studies of the effects of valerian have focused on the efficacy for its sedative actions in both humans and rodents. A meta-analysis of 16 clinical trials examining 1093 patients concluded that valerian might improve sleep quality without serious side effects (Bent, et al, 2006). A review of the human clinical trials examined 37 studies involving over 1900 patients concluded that valerian (up to 1800 mg) does not appear to significantly impair psychomotor or cognitive abilities, induce sedation or alter moods (Taibi et al., 2007). In these studies only mild adverse effects were reported, including headache, morning hangover, and GI complaints, nausea, and diarrhea. These effects were not different from those in patients taking either the placebos or benzodiazepines (Taibi et al 2007). There are six case reports of hepatotoxicity (MacGregor et al 1989; Bagheri et al., 1993; Cohen and Del Toro, 2006). These patients were taking mixtures of herbal supplements containing valerian and other herbs. The contribution of valerian to the hepatotoxicity is uncertain. Thus few studies have demonstrated adverse reactions to acute or short-term use of valerian.

Experimental Toxicity and Carcinogenicity Studies:

Acute studies

Few studies are available on the toxicity of valerian. There are several acute toxicity studies which demonstrate low oral toxicity (LD50 > 3 g/kg) following exposure to either valerian extracts, valtrate, or valeranone. However, most of these studies provide limited information on the study methods and no histopathology.

Valerian constituents, in particular the valepotriates, are mutagenic in *Salmonella typhimurium* TA100 and *Escherichia coli* WP2 and WP2 uvrA-. Increases in the frequency of micronuclei in polychromatic erythrocytes in the femur, increases in aberrations in chromosomes of the testis and spermatozoa abnormalities were observed in male mice receiving a valerian extract (500 – 2000 mg/kg/d) for 7 days (Al-Majed et al., 2006). These studies suggest that some of the constituents of valerian may be genotoxic.

There are limited reproductive and developmental studies available for valerian products. Administration of 2.79 g/kg from GD 1-8 or GD 8-15 of an ethanolic (45%) valerian extract produced no effects on reproductive endpoints such as live fetuses/litter and percent preimplantation loss (Yao et al 2007). Increases in the number of fetuses with retarded ossification were observed in rats treated with 12-14 mg/kg/d of a mixture of 80% didrovaltrate, 15% valtrate, and 5% acevaltrate from GD1-19 (Tufik et al 1994).

In vitro studies

In vitro studies suggest that valerian preparations induce neurobehavioral effects by binding to GABA receptors and/or activating adenosine receptors. Petroleum ether and dichloromethane extracts of valerian were found to be weakly estrogenic in MCF-7 cells.

Key Issues:

No chronic toxicity studies are available for valerian products and limited acute/subacute studies are available. Thus the potential toxicity of valerian is poorly characterized. Studies on valerian use suggest that women of reproductive age are taking this herb and may be a sensitive subpopulation.

Valerian products include pills, ground roots and extracts that range from 100% water to 70% ethanol. Thus initial screening methods are required to determine which valerian preparation would be best to evaluate.

Valerian is used as a sedative so one of the potential toxicities of this herb may involve its central nervous system (CNS) effects. Toxicities other than those related to the pharmacological effects of valerian may include developmental toxicities. There are two limited studies suggesting that valerian extracts retard ossification in rats and mice following developmental exposures. In addition, there are *in vitro* assays that report estrogenic activity of different valerian extracts. These results suggest that developmental toxicity studies are warranted.

Proposed Approach:

The first aim is to develop an approach to prioritize which valerian preparations to study. A second aim is to develop a tiered approach to testing valerian that provides guidance on what tests are of most value. One of the potential toxicities of valerian may be an extension of its pharmacological actions. Thus neurotoxicity would be an important endpoint to examine. An alternative hypothesis is that the toxicity is independent of its pharmacological effects, thus approaches that complement the neurotoxicity studies are necessary aims.

Specific Aim 1-Choosing the test material:

Since many extracts are sold based on valerenic acid content, one approach would be to chemically characterize the different extracts compared to their valerenic acid content. Since these extracts are likely to have different compositions, a second tier of screening is required.

Second tier screens would include *in vitro* screens examining binding to GABA receptors, which is a putative mechanism of action for its pharmacological actions. In addition, reports of estrogenic and mutagenic activity suggest that *in vitro* screens examining these endpoints should be included in the second tier screens.

If there are not clear differences in potency across the second tier screens, as a third tier, acute *in vivo* screens could be used. Decreases in motor activity are the most

common finding for valerian extracts and this may be a useful screen for determining which extract(s) to study. Uterotrophic assays could also be employed to evaluate the estrogenic activity of the different preparations.

Specific Aim 2-Toxicity Testing:

Tier 1 testing would include oral 14-day subacute, and 90-day subchronic studies in male and female rats and mice to characterize the toxicity of valerian. Special attention to behavioral toxicities will be included. Additionally, evaluate valerian for mutagenicity by conducting an *in vivo* micronucleus assay.

Tier 2 testing would include reproductive and developmental toxicity studies with special emphasis on neurotoxicity and landmarks of sexual maturation to investigate the biological implications of potential interactions with sex hormone receptors.

Tier 3 testing would include 2-year chronic toxicity studies in male and female rats and mice to evaluate the potential carcinogenic effects of valerian.

Significance and Expected Outcome:

Limited studies have examined the toxicity of valerian root preparations. The proposed studies will provide a tiered approach for evaluating the potential toxicity of this herbal product. This is particularly important due to the lack of adequate chronic and developmental toxicity studies for valerian. These studies would provide FDA with information that will allow for a better assessment of the safety of valerian.

References:

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